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Permalink
https://escholarship.org/uc/item/73f7r6vz

Journal
Journal of Clinical Microbiology, 38(7)

ISSN
0095-1137

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Publication Date
2000-08-07

Peer reviewed
Detection of *Leptospira* spp. in the Aqueous Humor of Horses with Naturally Acquired Recurrent Uveitis

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Received 12 November 1999/Returned for modification 26 February 2000/Accepted 30 April 2000

Leptospiral organisms have long been presumed to be associated with the presence of equine recurrent uveitis. This project was undertaken to determine the presence of *Leptospira* spp. in the aqueous humor of horses with uveitis to determine if there was an association with inflammation. Thirty horses were determined to have recurrent uveitis based on clinical evaluation or history. Sixteen horses were judged clinically and historically to be free of uveitis and were used as controls. Aqueous humor samples were cultured and evaluated by PCR for the presence of *Leptospira* DNA. Serum was collected and evaluated for the presence of antibodies against five serovars in a leptospirosis panel. Twenty-one of 30 horses with recurrent uveitis and one of 16 uveitis-free horses were positive by PCR for the presence of *Leptospira* DNA. Six of these 21 horses with uveitis were culture positive for *Leptospira* spp. from the aqueous humor. Serologic results did not correlate well with the presence of *Leptospira* DNA or organisms in the aqueous humor. *Leptospira* spp. are present in a high percentage of horses with naturally occurring recurrent uveitis.

Recurrent uveitis is a leading cause of blindness in horses worldwide. The signs (4, 17, 20) and lesions (3, 4, 10) illustrate the severity of the clinical course. The inflammation is non-granulomatous in nature and recurs at unpredictable intervals. The cornea, lens, vitreous body, and retina may also be involved. The inciting cause is usually not identified. Proposed causes include parasites, neoplasia, trauma, and infectious agents (4).

Leptospirosis has long been cited as a cause of equine recurrent uveitis, particularly *Leptospira interrogans* serovar pomona (1, 2, 8, 11, 20). Uveitis typically occurs months to years after naturally acquired (18, 19) or experimentally induced (15, 22) infection with serovar pomona. The purpose of this study was to determine the presence of *Leptospira* in the aqueous humor of horses with uveitis to determine if there was an association with inflammation.

**MATERIALS AND METHODS**

Thirty horses that ranged in age from 3 to 25 years (mean age, 13.7 years) were diagnosed with recurrent uveitis, either because they had lesions which were considered diagnostic of this syndrome or had a history of recurring episodes of uveitis. Sixteen horses that ranged in age from 2 to 15 years (mean age, 6.9 years) were selected as controls. These horses did not have any history of, or any oculocutaneous signs compatible with, recurrent uveitis. The gender ratios for the control group and horses with uveitis were similar (63% male, 37% female). Horses with uveitis were culture positive for *Leptospira* spp. from the aqueous humor. Thirty horses were determined to have naturally occurring recurrent uveitis.

The cornea, lens, vitreous body, and retina may also be in-

### Detection of PCR products.

For sample collection, some horses with uveitis were euthanatized with intravenous injections of xylazine (1.1 mg/kg of body weight) and ketamine (2.2 mg/kg).

The remainder as well as all the control horses were euthanatized with an overdose of sodium pentobarbital. Aqueous humor was collected with a 27-gauge butterfly catheter and a 3-ml syringe. About 1.5 ml was obtained from each eye. For 27 of the 30 horses with uveitis and 12 of the 16 control horses, about 0.3 ml of this aqueous sample was placed into a transport medium and then transferred to the modified Tween 80-albumin medium described previously (9) and cultured at 27°C. The remainder was frozen immediately for PCR evaluation. Blood was collected from 28 of the 30 horses with uveitis and all the control horses for serologic testing for *Leptospira* spp. The eyes were enucleated from the horses that had been euthanatized, placed in Bouin’s solution, and routinely prepared for microscopic examination.

Culture. Aqueous humor (0.3 to 0.6 ml) was inoculated into the transport medium, which consisted of 87 mg of KH₂PO₄ and 644 mg of Na₂PO₄·12H₂O and 1% bovine serum albumin. A 1:10 dilution of inoculated transport medium was then added to the culture medium, PLM-5 (Centeon, Kankakee, Ill.) along with 0.167% BBL agar and 200 μg of 5-fluorouracil/ml. Samples were incubated at 27°C for at least 2 months. Once a week, 5 μl of culture was viewed under an ×40 dark-field microscope for the presence of leptospires. A compact zone of growth approximately 1 cm from the meniscus was visible after 1 week in all positive cultures. The presence of *Leptospira* spp. in this zone was confirmed by microscopy.

Serology. The microscopic agglutination test was used to determine the sero-

### Conclusion

The aqueous humor was boiled for 10 min and centrifuged for 5 min, and the supernatant was stored at −20°C. PCR amplification for the presence of leptospiral DNA was performed in a total volume of 100 μl. Thirty microliters of supernatant was mixed with a solution consisting of sterile water, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 130 μM (each) dATP, dGTP, dCTP, and dTTP, 5 μM of each primer. Each sample was overlaid with 30 μl of sterile mineral oil to prevent evaporation.
eliquidation of missing values.

RESULTS

PCR. Twenty-one (70%) of the horses with uveitis (n = 30) had leptospiral DNA in the aqueous humor detectable with PCR. Fifty-five samples were collected from these horses, of which 30 (55%) were positive. Ten horses were positive bilaterally, and seven were positive unilaterally (23.5%). For four horses, either only one eye was present or permission was given to sample only one eye, and three of these eyes (75%) were positive. The association between a positive PCR result and uveitis was significant (P < 0.0011). In contrast, only one eye of the 29 eyes sampled (3.4%) from the 16 control horses (6.25%) was positive with PCR.

Culture. Of 27 horses with uveitis (22.2%) and none of 12 control horses tested were culture positive for Leptospira from the aqueous humor. The horses with positive cultures were also positive by PCR (eight of nine eyes). The association between a positive culture, however, and uveitis was not significant (P = 0.151) due to the low sensitivity of this test. Preliminary characterization of the isolates was performed by the National Leptospirosis Reference Center in Ames, Iowa. Four of the cultures were identified as serovar pomona. The other two isolates, though identical, did not match any of the serovars either serologically or genetically and thus are still unidentified.

Serology. Twenty-four of 28 horses with uveitis (85.7%) and 10 of 16 control horses (62.5%) had serologic titers for Leptospira of ≥1:100. Sixteen of the horses with uveitis (57.1%) and two of the control horses (12.5%) had titers of ≥1:400. By Fisher’s exact method and logistic regression, the positive titer for serovar pomona (≥1:100) was significant (P = 0.01 and 0.0001, respectively). The positive titers for pomona, gripotyphosa, and hardjo were significant by the Wilcoxon tests (P = 0.019, 0.039, and 0.04, respectively).

There was no significant association between the breed, gender, or age and the presence of uveitis.

DISCUSSION

Leptospiral DNA was detected in the aqueous humor of 21 of 30 horses with uveitis by PCR. Only that of 1 of 16 control horses was positive. Comparisons between antibody titers in aqueous humor and serum have led to the conclusion that an elevated aqueous humor titer suggests local antibody production, supporting a diagnosis of leptospira-associated uveitis (5; K. Schwink, Abstr. 21st Ann. Meet. Am. College Vet. Ophthalmol., p. 164–165, 1990). Although aqueous humor antibody titers were not determined as a comparison to serological values in this study, the presence of the organisms in the aqueous humor supports a diagnosis of leptospira-associated uveitis. The eye has been classically considered an immune-privileged site (12). Investigations for the presence of onchocerciasis, leptospirosis, toxoplasmosis, and brucellosis were frequently unrewarding (12). It had commonly been accepted that, although these diseases play a role in the initiation of ocular inflammation, subsequent development of hypersensitivity and autoimmune responses were responsible for the recurrence of the uveitis in the clinical cases and that evidence of the initial insult may be difficult to find (12).

By PCR assay, leptospiral DNA was found in the aqueous humor of 21 of 30 horses. This technology has been utilized for the rapid detection of leptospiral infections in cattle urine (7) and in blood, cerebrospinal fluid, urine, and aqueous humor from human beings (14, 16; F. Merien, P. Perolat, E. Mancel, D. Persan, and G. Baranton, Letter, J. Infect. Dis. 168:1335–1336, 1993). The diagnosis of leptospirosis had previously relied on culturing of the organism(s), serologic methods (microscopic agglutination technique), or dark-field microscopy, which is positive only at certain stages of the infection. Culturing of leptospires is often not successful, and it is slow and susceptible to contamination (16).

Serologic studies have been performed to evaluate the prevalence of leptospiral antibody titers in horses with and without uveitis (6, 13). Reliance on the use of serology as a means of establishing a diagnosis is uncertain, and opinions differ as to what constitutes a significant leptospiral titer. A positive serologic reaction does not necessarily indicate current or recent infection because titers may persist for more than 7 years after infection (21).

The positive serologic results of our study were not predictive of the aqueous PCR status. Two horses were positive by the aqueous humor PCR and culture but were serologically negative. Notwithstanding, the cultured isolates were not serologically reactive with any of the serovars used in the microscopic agglutination test and thus may reflect an as yet unidentified leptospiral serovar. This suggests that there is a need to expand the number of serovars which are being evaluated serologically or that some horses are not mounting a systemic immune response to these organisms.

Although leptospiral organisms are difficult to grow in culture media, 22% of the aqueous humor samples from horses with uveitis were culture positive in this study. Brem et al. (1, 2) have also reported the culturing of leptospires from horses with uveitis. It is important to note that all of the positive cultures were also positive by PCR. In this study it is apparent that PCR is a more reliable tool for detecting the presence of leptospires in equine recurrent uveitis.

We found no significant association between breed and uveitis, contrary to another study in which the Appaloosa was found to be at greater risk (6). This may have been due to the relatively small number of horses in our study.

ACKNOWLEDGMENTS

This research was supported by the University of California-Davis Center for Equine Health. We thank Annette Olson and the National Leptospirosis Reference Center for the assistance in this research.

REFERENCES